

What is claimed is:

1. A method for producing a cell comprising a modified foreign chromosome(s) or a fragment(s) thereof, which comprises the steps of:

- (a) preparing a microcell comprising a foreign chromosome(s) or a fragment(s) thereof, and transferring said foreign chromosome(s) or a fragment(s) thereof into a cell with high homologous recombination efficiency through its fusion with said microcell;
- (b) in said cell with high homologous recombination efficiency, inserting a targeting vector by homologous recombination into a desired site of said foreign chromosome(s) or a fragment(s) thereof and/or a desired site of a chromosome(s) derived from said cell with high homologous recombination efficiency, thereby marking the desired site; and
- (c) in said cell with high homologous recombination efficiency, causing deletion and/or translocation to occur at the marked site of said foreign chromosome(s) or a fragment(s) thereof.

2. The method of claim 1, wherein a plurality of said cells with high homologous recombination efficiency are subjected to whole cell fusion after steps (a) and (b) and are subjected to step (c).

3. The method of claim 2, wherein a plurality of said

cells with high homologous recombination efficiency each comprises a distinct foreign chromosome(s) or a fragment(s) thereof.

4. The method of claim 1, wherein said cell comprising a modified foreign chromosome(s) or a fragment(s) thereof is an animal cell.

5. The method of claim 4, wherein said animal cell is a mammalian cell.

6. The method of claim 4, wherein said animal cell is a non-human animal cell.

7. The method of claim 1, wherein said targeting vector contains a telomere sequence which is introduced into a desired site by insertion of the targeting vector.

8. The method of claim 2, wherein said deletion occurs at a site into which said telomere sequence has been introduced.

9. The method of claim 1, wherein said targeting vector comprises a recognition sequence for a site-directed recombination enzyme, and said recognition sequence is introduced into a desired site by insertion of the targeting vector.

10. The method of claim 9, wherein a vector, which is capable of expressing a site-directed recombination enzyme, is introduced into said cell with high homologous recombination efficiency simultaneously with or after insertion of said targeting vector comprising the recognition sequence for a site-directed recombination enzyme, so that an activity of said site-directed recombination enzyme is expressed, resulting in deletion and/or translocation of said foreign chromosome(s) or a fragment(s) thereof at a site in which said recognition sequence for a site-directed recombination enzyme is introduced.

11. The method of claim 10, wherein said translocation occurs between a plurality of foreign chromosomes or fragments thereof.

12. The method of claim 11, wherein a plurality of said foreign chromosomes are derived from the same species.

13. The method of claim 12, wherein said same species is a human.

14. The method of claim 11, wherein a plurality of said foreign chromosomes are derived from different species.

15. The method of claim 14, wherein said species are a

human and a mouse.

16. The method of claim 10, wherein said translocation occurs between a foreign chromosome(s) or a fragment(s) thereof and a chromosome(s) derived from said cell with high homologous recombination efficiency.

17. The method of claim 9, wherein said site-directed recombination enzyme is a Cre enzyme.

18. The method of claim 9, wherein said recognition sequence for a site-directed recombination enzyme is a LoxP sequence.

19. The method of claim 1, wherein said cell with high homologous recombination efficiency is an embryonic stem cell (or ES cell).

20. The method of claim 1, wherein said cell with high homologous recombination efficiency is a chicken DT-40 cell.

21. The method of claim 1, which further comprises a step of screening a cell comprising said foreign chromosome(s) or a fragment(s) thereof in which deletion and/or translocation has occurred.

22. The method of claim 21, wherein said screening is

based on expression of a marker gene.

23. The method of claim 22, wherein said marker gene is a drug-resistance gene.

24. The method of claim 22, said marker gene is a green fluorescent protein-encoding gene derived from the jellyfish *Aequorea victoria* or a modified gene thereof.

25. The method of claim 1, wherein said foreign chromosome(s) or a fragment(s) thereof is derived from a human.

26. A method for producing a chimeric non-human animal comprising a modified foreign chromosome(s) or a fragment(s) thereof, which comprises the steps of:

(a) preparing a microcell comprising a foreign chromosome(s) or a fragment(s) thereof, and transferring said foreign chromosome(s) or a fragment(s) thereof into a cell with high homologous recombination efficiency through its fusion with said microcell;

(b) in said cell with high homologous recombination efficiency, inserting a vector by homologous recombination into a desired site of said foreign chromosome(s) or a fragment(s) thereof, and/or a desired site of a chromosome(s) derived from said cell with high homologous recombination efficiency, thereby

marking said desired site;

(c) in said cell with high homologous recombination efficiency, causing deletion and/or translocation to occur at the marked site of said foreign chromosome(s) or a fragment(s) thereof; and

(d) preparing a microcell comprising said foreign chromosome(s) or a fragment(s) thereof in which deletion or translocation has occurred, and transferring said foreign chromosome(s) or a fragment(s) thereof into a pluripotent non-human animal cell through its fusion with said microcell.

27. The method of claim 26, wherein a plurality of said cells with high homologous recombination efficiency are subjected to whole cell fusion after steps (a) and (b) and are subjected to step (c).

28. The method of claim 27, wherein a plurality of said cells with high homologous recombination efficiency each comprise a distinct foreign chromosome(s) or a fragment(s) thereof.

29. The method of claim 26, wherein said targeting vector comprises a telomere sequence which is introduced into a desired site by insertion of the targeting vector.

30. The method of claim 29, wherein said deletion occurs at a site where said telomere sequence has been

introduced.

31. The method of claim 26, wherein said targeting vector comprises a recognition sequence for a site-directed recombination enzyme, and said recognition sequence is introduced into a desired site by insertion of the targeting vector.

32. The method of claim 31, wherein a vector, which is capable of expressing a site-directed recombination enzyme, is introduced into said cell with high homologous recombination efficiency simultaneously with or after insertion of said targeting vector comprising said recognition sequence for a site-directed recombination enzyme, so that an activity of said site-directed recombination enzyme is expressed, resulting in deletion and/or translocation of said foreign chromosome(s) or a fragment(s) thereof at a site into which said recognition sequence is introduced.

33. The method of claim 32, wherein said translocation occurs between a plurality of foreign chromosomes or fragments thereof.

34. The method of claim 32, wherein said translocation occurs between said foreign chromosome(s) or a fragment(s) thereof and said chromosome(s) derived from said cell with high homologous recombination

efficiency.

35. The method of claim 31, wherein said site-directed recombination enzyme is a Cre enzyme.

36. The method of claim 31, wherein said recognition sequence for site-directed recombination enzyme is a LoxP sequence.

37. The method of claim 26, wherein said cell with high homologous recombination efficiency is an embryonic stem cell (or ES cell).

38. The method of claim 26, wherein said cell with high homologous recombination efficiency is a chicken DT-40 cell.

39. The method of claim 26, which further comprises a step of screening cells comprising a foreign chromosome(s) or a fragment(s) thereof in which deletion and/or translocation has occurred.

40. The method of claim 39, wherein said screening is based on expression of a marker gene.

41. The method of claim 40, wherein said marker gene is a drug-resistance gene.

42. The method of claim 40, the marker gene is a green



fluorescent protein-encoding gene derived from the jellyfish *Aequorea victoria* or a modified gene thereof.

43. The method of claim 26, wherein in the step (d), a microcell is produced from said cell with high homologous recombination efficiency; said foreign chromosome(s) or a fragment(s) thereof, in which deletion and/or translocation has occurred is transferred into a CHO cell through its fusion with said microcell; a microcell is produced from the CHO cell; and then said foreign chromosome(s) or a fragment(s) thereof in which deletion and/or translocation has occurred is transferred into a pluripotent cell through its fusion with said microcell.

44. The method of claim 26, said pluripotent cell is an embryonic stem cell (or ES cell).

45. The method of claim 26, said foreign chromosome(s) or a fragment(s) thereof is derived from a human.

46. A method for producing a non-human animal comprising a modified foreign chromosome(s) or a fragment(s) thereof, which comprises the steps of:  
(a) preparing a microcell comprising a foreign chromosome(s) or a fragment(s) thereof, and  
transferring said foreign chromosome(s) or a fragment(s) thereof into a cell with high homologous

recombination efficiency thorough its fusion with said microcell;

(b) in said cell with high homologous recombination efficiency, inserting a vector by homologous recombination into a desired site of said foreign chromosome(s) or a fragment(s) thereof, and/or a desired site of a chromosome(s) derived from said cell with high homologous recombination efficiency, thereby marking said desired site;

(c) in said cell with high homologous recombination efficiency, causing deletion and/or translocation to occur at the marked site of said foreign chromosome(s) or a fragment(s) thereof;

(d) preparing a microcell comprising said foreign chromosome(s) or a fragment(s) thereof, in which deletion and/or translocation has occurred, and transferring said foreign chromosome(s) or a fragment(s) thereof into a cell derived from a non-human animal through its fusion with said microcell; and

(e) transplanting the nucleus of said cell derived from the non-human animal into an enucleated unfertilized egg derived from a homologous non-human animal of the same species.

47. The method of claim 46, wherein a plurality of said cells with high homologous recombination efficiency are subjected to whole cell fusion after steps (a) and (b) and are subjected to the step (c).

48. The method of claim 47, wherein a plurality of said cells with high homologous recombination efficiency comprise a distinct foreign chromosome(s) or a fragment(s) thereof.

49. The method of claim 46, wherein said targeting vector comprises a telomere sequence, which is introduced into a desired site by insertion of the targeting vector.

50. The method of claim 49, wherein said deletion occurs at a site into which a telomere sequence has been introduced.

51. The method of claim 46, wherein said targeting vector comprises a recognition sequence for site-directed recombination enzyme, and said recognition sequence is introduced into a desired site by insertion of the targeting vector.

52. The method of claim 51, wherein a vector, which is capable of expressing a site-directed recombination enzyme, is introduced into said cell with high homologous recombination efficiency simultaneously with or after insertion of said targeting vector comprising said recognition sequence for a site-directed recombination enzyme, so that an activity of said site-directed recombination enzyme is expressed,

resulting in deletion and/or a translocation of said foreign chromosome(s) or fragment(s) thereof at a site into which said recognition sequence is introduced.

53. The method of claim 52, wherein said translocation occurs between a plurality of foreign chromosomes or fragments thereof.

54. The method of claim 52, wherein said translocation occurs between said foreign chromosome(s) or a fragment(s) thereof and said chromosome derived from a cell with high homologous recombination efficiency.

55. The method of claim 51, wherein said site-directed recombination enzyme is a Cre enzyme.

56. The method of claim 51, wherein said recognition sequence for a site-directed recombination enzyme is a LoxP sequence.

57. The method of claim 46, wherein said cell with high homologous recombination efficiency is an embryonic stem cell (or ES cell).

58. The method of claim 46, wherein said cell with high homologous recombination efficiency is a chicken DT-40 cell.

59. The method of claim 46, which further comprises a

step of screening cells containing a foreign chromosome(s) or a fragment(s) thereof in which deletion and/or translocation has occurred.

60. The method of claim 59, wherein said screening is based on expression of a marker gene.

61. The method of claim 60, wherein said marker gene is a drug-resistant gene.

62. The method of claim 60, wherein said marker gene is a green fluorescent protein-encoding gene derived from the jellyfish *Aequorea victoria* or a modified gene thereof.

63. The method of claim 46, wherein, in the step (d), a microcell is produced from said cell with high homologous recombination efficiency; said foreign chromosome(s) or fragment(s) thereof, in which deletion and/or translocation have/has occurred, is/are transferred into a CHO cell through its fusion with the microcell; a microcell is produced from the CHO cell; and then said foreign chromosome(s) or a fragment(s) thereof, in which deletion and/or translocation has occurred, is transferred into a cell derived from a non-human animal through its fusion with the microcell.

64. The method of claim 46, said cell derived from a

non-human animal is a culture cell derived from an embryo or a blastocyst.

65. The method of claim 46, said cell derived from a non-human animal is a culture cell derived from a fetus or an adult.

66. The method of claim 46, said cell derived from a non-human animal is a fibroblast cell derived from fetus.

67. The method of claim 46, said foreign chromosome(s) or a fragment(s) thereof is derived from a human.

68. A non-human animal, which retains a chromosomal fragment(s) obtained by deletion of a foreign chromosome(s) or a fragment(s) thereof.

69. The non-human animal of claim 68, wherein said chromosomal fragment(s) comprises:

- (i) a marker gene and a telomere sequence, and/or
- (ii) a recognition sequence for a site-directed recombination enzyme.

70. A non-human animal, comprising a recombinant foreign chromosome(s) obtained by translocation between a plurality of foreign chromosomes or fragments thereof.

71. The non-human animal of claim 70, wherein said recombinant chromosomal fragment(s) comprises:

- (i) a marker gene and a telomere sequence; and/or
- (ii) a recognition sequence for a site-directed recombination enzyme.

72. The non-human animal of claim 70, wherein said recombinant foreign chromosome(s) is independently maintained in the nucleus of the non-human animal cell.

73. The non-human animal of claim 70, wherein said recombinant foreign chromosome(s) is derived from a human.

74. The non-human animal of claim 70, wherein the recombinant foreign chromosome(s) is derived from human chromosomes #14 and #2.

75. The non-human animal of claim 70, wherein said recombinant foreign chromosome(s) is derived from human chromosomes #14 and #22

76. The non-human animal of claim 70, wherein said recombinant foreign chromosome(s) comprises genes for a heavy-chain and a light-chain  $\lambda$  of a human antibody.

77. The non-human animal of claim 70, wherein said recombinant foreign chromosome(s) comprises genes for a heavy-chain and a light-chain  $\kappa$  gene of a human

antibody.

78. The non-human animal of claim 70, which is a mouse.

79. The non-human animal of claim 70, which is an ungulata.

80. The non-human animal of claim 70, which is a bovine.

81. The non-human animal of claim 70, which is an ovine.

82. The non-human animal of claim 70, which is an avian.

83. The non-human animal of claim 70, which is a chicken.

84. A cell, comprising a recombinant chromosome(s) or a fragment(s) thereof obtained by deletion and/or translocation of a chromosome(s) or a fragment(s) thereof, which comprises at least a part of a human chromosome and into which  
(i) a marker gene and telomere sequence, and/or  
(ii) a recognition sequence for site-directed recombination enzyme is/are introduced.

85. A method for modifying a foreign chromosome(s) or a



fragment(s) thereof in a cell, which comprises the steps of:

(a) preparing a microcell containing a foreign chromosome(s) or a fragment(s) thereof, and transferring said foreign chromosome(s) or a fragment(s) thereof into a cell with high homologous recombination efficiency through its fusion with the microcell;

(b) in said cell with high homologous recombination efficiency, inserting a targeting vector by homologous recombination into a desired site of said foreign chromosome(s) or a fragment(s) thereof and/or a desired site of a chromosome(s) derived from said cell with high homologous recombination efficiency, thereby marking said desired site; and

(c) in said cell with high homologous recombination efficiency, causing deletion or translocation to occur at the marked site of said foreign chromosome(s) or a fragment(s) thereof.

86. An artificial chromosome vector, which comprises a centromere sequence derived from a human chromosome #14 or #21, and a recognition sequence for a site-directed recombination enzyme.

87. The artificial chromosome vector of claim 86, wherein said recognition sequence for a site-directed recombination enzyme is a LoxP sequence.

88. A recombinant chromosome or a fragment thereof obtained by deletion and/or translocation of a chromosome(s) or a fragment(s) thereof, into which (i) a marker gene and a telomere sequence, and/or (ii) a recognition sequence for a site-directed recombination enzyme is/are introduced, and which comprises at least a part of a human chromosome.

89. The recombinant chromosome or a fragment thereof of claim 88, which comprises fragments of human chromosomes #14 and #22.

90. The recombinant chromosome or a fragment thereof of claim 88, which comprises fragments of human chromosomes #14 and #2.

91. The recombinant chromosome or a fragment thereof of claim 88, which comprises genes for a heavy-chain and a light-chain  $\lambda$  of a human antibody.

92. The recombinant chromosome or a fragment thereof of claim 88, which comprises genes for a heavy-chain and a light-chain  $\kappa$  of a human antibody.

APP B1)